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Radiological and microbiological markers of invasive aspergillosis

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1) Introduction

1.1 Invasive fungal infections (IFI)

Fungi are eukaryotic organisms including yeasts and molds. Fungi are present in the environment and disseminate via cell budding or spore formation. Spores can be inhaled and cause primary lung diseases. Some yeasts (e.g.: *Candida* spp.) can also colonize the skin and gastro-intestinal tract and infect human by invasion of blood and tissues.
Some fungi cause endemic diseases (e.g. *Histoplasma capsulatum*), while others cause opportunistic infections in immunosuppressed patients (e.g. *Candida* spp., *Aspergillus* spp, *Mucorales*).

The evolution of modern medicine with the development of new treatments has improved the prognosis and life expectancy of many diseases. However, some treatments, such as anti-cancer chemotherapies and immunosuppressive drugs used in transplantation medicine, make the patients more susceptible to develop severe infectious diseases such as invasive fungal diseases [1]. The 12-month cumulative incidence of invasive fungal infections (IFI) in bone marrow and solid-organ transplant recipients is about 8% [2, 3]. Patients with hematologic cancers (e.g. acute leukemias) have a similar high risk to develop IFI during the neutropenic phase following myeloablative chemotherapy. Invasive aspergillosis is the most frequent IFI in these settings, followed by invasive candidiasis and mucormycosis.

1.2 Invasive aspergillosis

a) Pathogenicity
Aspergillus is a filamentous fungus, which comes from ancient lineages present since 1 billion years. It is the most ubiquitous fungus and its spores are present in the air and soil. We are continuously exposed to this fungus [4]. The spores are inhaled and reach the lungs alveoli. The immune system plays an indispensible role by preventing the infection. Respiratory epithelial cells as well as alveolar macrophages constitute the first barrier and defense against *Aspergillus* [5]. After fungal germination, the neutrophils are the dominant barriers against hyphae. There are several cell receptors, such as pathogen recognitions receptors (PPRs) that recognize some specific cell walls motifs and are able to release cytokines and chemokines and activate the neutrophils [4]. The NADPH oxidase present in phagocytes converts oxygen to superoxide anion, and is essential against bacterial and fungal infection. Patients with NADPH oxidase deficiency such as those with chronic granulomatous disease are extremely susceptible of developing an invasive aspergillosis. Other groups of patients at risk include hematologic cancer patients, allogeneic hematopoietic stem transplant (HSCT) cell recipients, solid-organ transplant (SOT) recipients and other patients receiving long-term corticosteroid or immunosuppressive therapies. The disease primarily affects

the lungs or sinuses and can disseminate to other organs (brain, skin, kidney, gastro-intestinal tract).
Aspergillus fumigatus is responsible for 50%-90% of human infections [2, 3, 6], while other species (*A. flavus*, *A. niger*, *A. terreus*) may occasionally cause diseases.

b) Epidemiology
Invasive Aspergillosis (IA) remains the most common mold infection in immunocompromised patients [2, 3]. It can be said that their prevalence has risen in the past few years. A retrospective cohort study made at the Fred Hutchinson Cancer Research Center in Seattle from 1985 through 1999, among hematopoietic stem cell transplantation (HSCT) patients, showed that the incidence of IA strongly increased after 1992 [7]. Another retrospective study was made in 11 Italian transplantations centers between 1999 and 2003 among HSCT patients. A total of 3228 patients were included in the study where 121 had an IFI (3,7%). The most frequent pathogen found was *Aspergillus* species (86 episodes) with a high mortality rate, followed by *Candida* species (30 episodes) [8]. In the United States, the Transplant-Associated Surveillance Network (TRANSNET) reported a 12-month cumulative incidence of IA among HSCT and SOT recipients of 1.6% and 0.7%, respectively [2, 3]. IA was the first and second cause of invasive fungal infection, respectively.

c) Prognosis and Mortality
In the TRANSNET cohorts, the 12-month survival rate of IA was 25% and 59% in HSCT and SOT patients, respectively [2, 3]. In another North-American cohort (PATH Alliance Registry), the 12-month survival of IA was 64% [6].

d) Clinical presentations
IA mostly involves the lungs, reflecting the most common portal of entry. Signs and symptoms, such as fever, cough and dyspnea are non-specific and most patients with IA do not have respiratory symptoms [4, 5]. Primary involvement of upper respiratory airways with sinusitis is also possible. In patients with severely impaired immune defenses, such as neutropenia, angio-invasion occurs and is characterized by a necrosis in the lung parenchyma. Pleuritic chest pains as well as hemoptysis are frequent symptoms. It can be complicated by dissemination in blood and infection of other organs such as skin, brain, kidneys, liver or spleen. If the central nervous system is involved, the consequences are dramatic, with seizures, focal neurologic symptoms or stroke [9].

e) Diagnosis
The diagnosis of IA is based on clinical, microbiological and radiological criteria. Unfortunately, the available diagnostic tests have limited performance. Clinical signs are often absent or delayed, radiological signs are not specific and microbiological tests lack sensitivity.

The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of

Allergy and Infectious Diseases Mycoses Study Group (MSG) published standard definitions for invasive fungal infections (IFI) to simplify clinical and epidemiological research [10]. The definition classifies the patients in 3 different groups of probability for the diagnosis of IFI, “proven”, “probable”, and “possible” IFI.

Proven IFI: Requires proof by histopathology or isolation of the fungus by culture of a normally sterile body site (e.g. blood, brain tissue…).
Probable IFI: Requires the presence of a host factor (immunosuppressive conditions), a radiological criterion of the disease (e.g. nodular lesion of the lung) and a mycological criterion (microbiological documentation of the fungus by culture, PCR or serological tests)
Possible IFI: This category only considers patients with host factors and radiological criteria of IFI but who do not have any mycological criterion.

Imaging technologies

It has been recognized since the 1980s that imaging plays a predominant role in the early diagnosis of IA [11]. High resolution CT-scanning represents the imaging of choice for the diagnosis of invasive fungal infections. Although the images are not specific and can occur in other infections or diseases, some of them are strongly suggestive of IA when observed in high-risk immunocompromised patients [12]. Well-circumscribed lung lesions (nodules or masses) represent the most frequent representation of IA among neutropenic patients [13]. Macronodules have more than 1cm diameter and small nodules <1cm. The halo sign corresponds to a central dense lesion surrounded by an area of ground glass opacity, which corresponds to a coagulation necrosis surrounded by hemorrhage. This sign occurs early in the course of IA (usually during the the first two weeks) [13, 14]. It has been recognized that the initiation of antifungal treatment on the basis of the identification of a halo sign is associated with a better response to treatment and improved survival [15]. Ground-glass-opacity is a hazy increased attenuation of the lung, through which the background of underlying bronchovascular structures is still visible, which is not the case with consolidation. The air crescent sign is a pocket of air interposed between a lung sequestrum. It occurs late in the course of the infection and is often followed by a cavitation. It may move when the patient changes the position and can cause hemoptysis if symptomatic [16]. Hypodense sign describes a central hypointensity in lung consolidation or nodules [17]. Its sensitivity is low, but the high specificity makes it a good predictor for IA, and could also be a precursor to the development of cavitation, which is a late sign of IA.

Microbiological diagnosis

The diagnosis of IA relies on a constellation of several diagnostic tests, including direct examination (e.g. silver staining of respiratory secretions or tissues), histopathology, culture, antigen detection (galactomannan, 1,3-beta-d-glucan) and PCR. Because the sensitivity and specificity of these markers is limited, these tests should be combined and interpreted in conjunction with

the underlying host conditions (underlying disease and immunosuppression) and clinical signs (mainly radiological signs).

Galactomannan (GM):
GM is a polysaccharide cell-wall component that is released during IA. It can be detected in serum, plasma or other body fluids by the Platelia *Aspergillus* enzyme immunoassay, a double sandwich ELISA using a monoclonal rat antibody directed against GM. The results of the test are expressed as a ratio of optical density (OD) with a threshold of 0,5 [18]. Two consecutive positive results above 0.5 increase the specificity. A screening strategy with GM monitoring once or twice a week is recommended for the early detection of IA in high-risk patients. False positive GM results have been reported, for example cross-reactivity with other fungi (e.g. *Penicillium*, *Fusarium*), β-lactam antibiotics, blood transfusions and blood-derived products or food additives (sodium gluconate) [18]. The performance of the GM test for the diagnosis of IA has been studied in two meta-analyses, with a sensitivity of 70-80% and a specificity of 80-85% [19, 20]. However, the results of the test showed differences among the different populations groups. Tests performances were acceptable among patients with hematologic malignancies, but they appears to be lower in solid-organ transplant recipients [20]. GM can be also detected in bronchoalveolar lavage fluid (BAL) or cerebrospinal fluid (CSF). In BAL, the test has a sensitivity and specificity of 85-90% and 90-95%, respectively [21, 22]. However, the optimal cutoff of GM in the BAL might be higher (≥1 instead of 0,5). One study also reported a good performance for detecting cerebral aspergillosis in CSF (88% sensitivity and 96% specificity) [23].

1,3-beta-d-glucan (BG)

Beta-D-glucan is a major polysaccharidic component of the fungal cell wall. It can be detected in serum in many invasive fungal infections, including IA, invasive candidiasis and *Pneumocystis jirovecii* pneumonia, with the exceptions of mucormycosis and cryptococcosis. Four (1-3)-β-d-glucan tests have been developed for BG detection in serum and their performance is similar [18]. The Fungitell test (Associates of Cape Cod Inc.) is the most widely used. The cutoff of positivity differs according to the test used and is 80 pg/ml for the Fungitell. False positive results have been reported in patients treated with B-lactam antibiotics, concomitant bacterial infections, renal replacement therapy, blood transfusions or blood derived products, immunoglobulins, as well as cellulose containing dressings [18]. A limitation of the test is the low sensitivity. A meta-analysis was performed among hemato-oncological patients including six cohort studies and showed a sensitivity of 49,6% and a specificity of 98,9% for two consecutive positive tests in serum [24]. The test is not suitable for testing in BAL or other clinical samples.

Histopathology and cytology
Respiratory secretions and any tissue biopsy should be analyzed by direct examination. A biopsy is not always possible because the patients are often very susceptible to bleeding and infections due to immunosuppression (e.g. thrombopenia in hematologic cancer patients), but it should nevertheless be

performed whenever possible. The demonstration of fungal elements invading tissues constitutes a criterion for proven IFI.

Culture

Culture of BAL or other respiratory secretions is important for the diagnosis. However, the sensitivity of BAL culture is low, ranging between 20% and 50% [6, 25, 26]. Furthermore, several cases of positive BAL culture reflect colonization rather than infection, especially in case of lung transplant recipients or patients with chronic lung diseases [27].

Culture should also be performed on any biological sample taken from a suspected site of infection (skin, tissue biopsy, pleural fluid).

In blood culture, it is extremely difficult to find molds, because they do not sporulate in blood.

Polymerase chain reaction (PCR)

PCR will be included in the next revised EORTC/MSG definitions of IA. Lack of availability and lack of standardized methods however remains a limitation. Indeed, we can find a lot of differences concerning the gene targets, the choice of primers and probes, the clinical specimens tested (blood, BAL fluid, serum, CSF, tissue biopsy), the thresholds of detection, and the positive cut-off [28]. A meta-analysis conducted among onco-hematological patients showed a sensitivity of 75% and a specificity of 87% for two PCR positive results in blood or serum, and a sensitivity of 88% and a specificity of 75% for only one positive result [29]. The results found in BAL are even more encouraging with a sensitivity of 91% and a specificity of 92% [30]. The studies that can be found in literature mostly include onco-haematological patients. The performance of PCR in patients with other type of immunosuppression is unknown. Review and meta-analysis comparing the PCR and GM in serum or BAL showed encouraging results when combining both diagnostic methods [31-33]. Recent development of a consortium for improved standardization of PCR procedures motivates the introduction of *Aspergillus* PCR testing in the ongoing revised definitions of invasive fungal disease from the EORTC/MSG [34].

f) Treatment and prevention

Three antifungal drug classes are available for the treatment of IA, the polyenes (e.g. amphotericin B) the triazoles (e.g. voriconazole, itraconazole, posaconazole and isavuconazole) targeting the ergosterol component of the cell membrane, and the echinocandins (e.g. caspofungin, micafungin, anidulafungin) targeting the beta-glucan component of the fungal cell wall.

The primary therapy for IA is voriconazole according to the guidelines of the Infectious Diseases Society of America [35]. In a randomized, unblinded trial, initial treatment with voriconazole showed better outcomes than deoxycholate amphotericin B (52,8% success in the voriconazole group vs 31,6% in the amphotericin B group) [36]. Survival was also significantly better in the voriconazole group (70,8%) vs the amphotericin B group (57.9%) and voriconazole therapy was associated with fewer side effects. New

formulations of Amphotericin B (liposomal or lipid-complex) and echinocandins are second-line options [35].
Besides treatment of proven or probable IA cases, strategies have been developed for the prevention of IA. The first approach is universal posaconazole prophylaxis for all hematologic cancer patients during the high-risk neutropenic phase [37, 38]. Another strategy consists in a preemptive approach with monitoring of fungal biomarkers in serum, such as galactomannan or beta-glucan, and start further investigations (e.g. CT-scan, bronchoscopy) and antifungal treatment in case of a positive test.

2) Objectives

The isolation ward of Lausanne University Hospital applies a standardized preemptive approach for the diagnosis and management of IA for all patients with hematologic cancer and prolonged neutropenia following intensive chemotherapy, who are at high risk to develop such infections. Of note, instead of giving systematic posaconazole prophylaxis to all patients, we monitor twice weekly the galactomannan (GM) in serum. Chest CT-scan is performed for any clinical suspicion (e.g. positive GM value, persistent or relapsing fever) and bronchoscopy with analysis of the bronchoalveolar lavage fluid (BAL) is performed in case of abnormal chest CT imaging.

The general aim was to investigate the respective contribution and timing of each of these diagnostic procedures (i.e. serum GM, CT-scan, bronchoscopy) for the diagnosis of IA. In particular, the following objectives have been defined:

i) **To assess the role of galactomannan (GM) monitoring in serum for the early diagnosis of IA**.
Serum GM testing is recommended for the diagnosis of IA [28, 35]. For high-risk hematologic cancer patients, a monitoring of GM in serum (e.g. bi-weekly) is recommended during the neutropenic phase following intensive myeloablative chemotherapy. However, the actual benefit of serial GM monitoring for the early detection of IA is not well demonstrated with controversial data reported in the literature [39, 40]. The objective was to determine the role of serum GM monitoring for the early detection of IA (timing of positivity of serum GM compared to detection of IA signs by CT-scan). A cost-effectiveness analysis of serum GM monitoring was also performed.

ii) **To assess the different radiological patterns of IA in this patients population**

Radiological signs of IA are not specific. Well-circumscribed lesions (i.e. nodules with or without halo sign) are considered to be highly suggestive of IA in this patients' population. However, other radiological patterns, such as non-specific density or infiltration, pleural effusion or tree-in-bud patterns may be observed and their frequency is not well described in the literature. The objective was to provide a systematic description of all lung lesions observed in IA cases.

3) Methods

All patients admitted in the Isolation Ward of Medicine of the Lausanne University Hospital for a chemotherapy course for the treatment of hematologic malignancies between January 2007 and November 2017 were considered for this analysis. Patients with a diagnosis of proven or probable invasive mold infections according to EORTC-MSG criteria [10] were identified via our electronic database system (Secutrial). Clinical data were retrospectively collected in the electronic medical records of these patients, including baseline characteristics (gender, age, underlying malignancy, other underlying diseases, type of chemotherapy), characteristics of invasive fungal infections (EORTC-MSG classification, type of pathogenic mold, site of infection), results and timing of diagnostic procedures (galactomannan in serum and BAL, CT-scan, bronchoscopy, other invasive procedures, fungal cultures and PCR) and antifungal treatment (antifungal drug and timing of introduction). Response to the therapy was assessed at week 4 according to EORTC-MSG definitions of success or failure [41]. Overall survival was assessed at week 12.

Inclusion criteria:

This analysis was restricted to patients presenting the following criteria:

- Patients with hematologic cancer, patients with an expected duration of neutropenia of more than 10 days, for whom a monitoring of serum galactomannan (GM) was performed bi-weekly during the neutropenic phase. This usually includes:
    - Patients with acute leukemia undergoing intensive myeloablative chemotherapy
    - Patients with aplastic anemia and prolonged neutropenia
- Diagnosis of proven or probable IA according to EORTC-MSG criteria

Exclusion criteria:

- Patients with other types of hematologic cancers, for whom other chemotherapies were used with an expected duration of neutropenia of less than 10 days and no monitoring of GM in serum.
- Diagnosis of proven or probable invasive fungal infections other than IA or mixed (i.e. *Aspergillus* spp. and non-*Aspergillus* fungi).

Aim i: To assess the role of galactomannan monitoring in serum for the early diagnosis of IA

In order to assess the actual role of GM monitoring in serum for the early detection of IA, patients were stratified in three groups:

1) Group GM: the first marker of IA was a positive serum GM and CT-scan (or other diagnostic procedures) confirmed the diagnosis of proven/probable IA
2) Group CT: the first marker of IA was an abnormal radiological finding on a CT-scan, that was motivated by persistent fever or clinical signs, and positive GM testing in serum subsequently confirmed the diagnosis of probable IA
3) Group IP: the first marker of IA was an abnormal radiological finding on a CT-scan and invasive procedure, such as bronchoscopy or tissue biopsy, was required for the diagnosis of proven/probable IA, while serum GM remained negative

For the cost-effectiveness analysis of serum GM monitoring, we calculated the total number of hospital stays and neutropenic days for the patients eligible during the study period in order to determine the number of GM tests (and related costs) required for the early detection of a single IA case.

Aim ii: To assess the different radiological patterns of IA in this population

Chest CT-scan of all patients were analyzed with an expert radiologist (Dr. Catherine Beigelmann) who did not know the characteristics of the patients. A clinical report form was fulfilled with identification of the following distinct radiological patterns: nodules (number, size), halo sign, reversed halo sign, hypodensity, air-crescent sign, cavity, tree-in-bud pattern, alveolar density, non-specific infiltrate, ground-glass opacity, pleural effusion, extra-pulmonary lesions. The proportion of each sign was assessed.

4) Results

A total number of 44 proven or probable invasive fungal infections (IFI) were identified over the 10-year period. Cases of IFI other than IA or mixed (n=9) were excluded. We also excluded 5 additional cases for which GM monitoring in serum was not performed. Thus, a total of 30 IA cases were considered for this analysis.

Characteristics of IA cases **(Table 1):**

These 30 IA cases (6 proven, 24 probable) were diagnosed in 30 patients (21 acute myeloid leukemias, 4 acute lymphoblastic leukemias, 5 other hematologic malignancies). The primary site of infection was the lung in 28 cases (93%) and an extra-pulmonary site in 2 cases (1 rhinosinusal and 1 cerebral aspergillosis). CT signs consistent with IA were found in all cases and bronchoscopy with BAL was performed in 24/28 (86%) pulmonary IA.

Aspergillus fumigatus was documented in 14 (47%) and other *Aspergillus* spp. in 3 cases (10%), while diagnosis relied on a positive GM test only in 13 cases (43%).

***Respective contributions of the different fungal markers for the diagnosis of IA* (Table 1):**
The respective contribution of each mycological marker is shown in Table 1. Overall, a positive GM in serum and/or BAL were found in 26 cases (87%), while culture and PCR added a modest contribution in identifying the 4 (13%) remaining cases that were GM negative. A positive culture of respiratory samples was obtained in only 5 cases (17%). PCR was positive in 11/24 (46%) BAL and, overall, allowed species identification in 12 cases (40%). A positive GM in serum was found in 15 cases (50%), of which 11 (73%) had at least two consecutive positive values. A positive GM in BAL was found in 17 of 24 patients (71%) who underwent bronchoscopy (median optical density index 2.9, range 0.6 – 6.7). Direct examination (silver staining) of BAL was negative in all cases and histopathologic examination of tissue biopsy found mycelia in 4 cases (2 sinus and 2 lung biopsies).

***Respective contributions of GM, CT and invasive procedures (IP) for IA diagnosis* (Table 3):**
Positive GM in serum was the first indicator of IA triggering further investigations (GM group) in 10 cases (31%). In 22 cases (69%), IA was first suspected on the basis of abnormal CT finding, while GM in serum was negative. A subsequent positive GM in serum led to the diagnosis of probable IA in 6 cases (19%) (CT group). In the remaining 16 cases (50%), bronchoscopy or other invasive procedures were required to achieve diagnosis of at least one probable IA by positive GM in BAL, culture or PCR (IP group)

Response to therapy and outcome:
Response to therapy at week 4 and overall survival rate at week 12 was significantly better for patients with negative serum GM compared to those with a positive GM (100% vs 54%, p=0.005 and 100% vs 57%, p=0.006). However, among patients with a positive serum GM, outcome was similar between GM and CT groups (Table 3).

Cost-effectiveness analysis of galactomannan (GM) screening:
Considering that bi-weekly GM monitoring was useful for the early detection of IA in 10/32 cases (31%) (GM group), we assessed the number of tests required for the detection of one IA case.
A total of 268 patients were admitted for induction or consolidation chemotherapy of acute leukemia during the study period for a total number of 17’545 days of neutropenia.
Although the exact number of GM tests performed is not available, we estimated that a total of 5’010 tests were required to monitor GM bi-weekly during the neutropenic phase of these patients.

As 10 IA cases were identified by this approach, we estimated that approximately 500 GM tests are required for the early detection of a single IA case, which represents a total cost of 14'500 CHF (29CHF/test) for one case.

Radiological signs of invasive fungal infections (IFI) on chest CT **(Table 4):**
For this analysis, only the 28 patients with pulmonary IA were considered. While the characteristic nodules (i.e. well circumscribed lesions) were present in most cases (n=24, 86%), we observed a very large diversity of clinical radiological signs in the setting of IA, as illustrated in Table 4.
The halo sign, which is recognized as an early marker of IA, was observed in 21/24 cases (75%) with nodular presentation, suggesting that most cases were detected at an early stage. Other radiological signs that are not characteristic of IA in this population, such as alveolar condensation or tree-in-bud patterns, were observed in a substantial proportion of cases.

5) Discussion

In this retrospective analysis, we analyzed all IA cases occurring among patients with hematologic cancer and prolonged neutropenia following intensive chemotherapy over a 10-year period. A total of 30 cases were found in 268 patients (incidence: 11%).
Our analysis focused on the diagnostic approach of invasive aspergillosis, which is particularly difficult and challenging.
The individual performance of GM in serum and BAL were of respectively 50% and 71%, which is relatively lower compared to previous literature data. Recent meta-analyses pooling results from multiple studies showed a 70-80% sensitivity for GM in serum and a 90% sensitivity in BAL [19-21]. The systematic use of bronchoscopy for documentation of possible IA may actually have increased the number of IA cases with negative serum GM. However, we also observed a substantial number of pulmonary IA with positive serum GM (at least two consecutive values) and negative GM in BAL. This is not in agreement with the study of D'Haese et al. suggesting that a negative GM in BAL has very high negative predictive value and virtually excludes IA [42]. Our results suggest that false negative results in BAL may be more frequently observed. However, despite these decreased sensitivity of individual GM testing, the combination of GM in serum and BAL allowed identification of most cases of this study. PCR testing in BAL had lower sensitivity (only 46%) compared to GM and added a modest value added in identifying a few more cases, while the yield of direct examination and fungal cultures remained desperately low.

The most relevant result of this study is the cost-effectiveness analysis of serial serum galactomannan monitoring in this population. Screening of GM in serum is commonly admitted as a pre-emptive diagnostic approach for early detection of IA. This strategy is supported by several studies showing that the diagnosis of IA based on GM detection in serum usually precedes diagnosis

by radiological signs on CT-scan [39, 43]. However, controversial data supporting the preeminence of CT over GM have also been published [40]. In our study, positive GM in serum was the first indicator of IA triggering further investigations in 10 cases (31%). We also analyzed the cost-effectiveness of GM monitoring and found that about 500 GM tests were required to detect a single IA, which represents a cost of 14'500 CHF. Based on these results, we may ask ourselves if the screening of GM two times a week makes a real difference for the early diagnosis of IA. On the one hand, this screening represents a substantial workload in terms of time and personal. On the other hand, IA is a very serious disease and important cause of mortality in cases of late detection and GM screening remains a relatively inexpensive test. Finally, we observed that the 10 patients who benefited from early detection of IA by a positive GM triggering further investigations (GM group) did not have a better outcome. While the goal of GM monitoring is to anticipate IA diagnosis for prompt start of antifungal therapy and possible improved outcomes, we observed that mortality was still high among these patients despite early GM detection. Actually, a positive GM test was a factor of bad prognosis irrespective of the timing of GM positivity with respect to CT, which probably reflects the degree of angio-invasion and dissemination of the disease. On the contrary, the prognosis was excellent for patients with negative GM and whose diagnosis of IA relied uniquely on the results of bronchoscopy (100% response at 4 weeks and 100% survival at 12 weeks). In reality bronchoscopy was necessary to achieve the diagnosis of proven/probable IA in half of the cases of this study. These results support our invasive diagnostic approach with systematic bronchoscopy for any suspicion of pulmonary IA on the basis of radiology. It is important to highlight that bronchoscopy was safe and not associated with complications during this 10-year period.
We thus concluded that, while GM remains the most efficient diagnostic marker of IA, the bi-weekly conventional pre-emptive approach of serum GM monitoring in high-risk neutropenic patients did demonstrate a modest benefit in terms of early diagnosis and no benefit in terms of improved outcomes. A clinical based approach with CT and punctual serum GM testing at key time points during the neutropenic phase (e.g. in case of persistent or relapsing fever), followed by systematic bronchoscopy in case of consistent CT lesions, may be an alternative approach.

Another aim of the study was to analyze the radiological pattern of IA. The role of CT-scan in the early detection of IA is well known. Suggestive radiological findings are often the first sign of IA [40]. The difficulty is that clinical signs of IA are not specific or absent in the early stage of the disease. The lesions are not pathognomonic and can be found in other IFI. Many publications have described the radiological pattern of IA in cancer neutropenic patients. Well-circumscribed lung lesions (nodules or masses) represented the most frequent radiological presentation of IA and the halo sign was usually present at early stages [12-15]. Our results confirmed the preeminence of nodules (86% of cases) and of the halo sign (present for 88% of them). However, we observed a large variety of other radiological signs that are usually not considered as radiological criteria of IA by the EORTC-MSG definitions [10]. For instance, tree-in-bud patterns or alveolar condensations were observed in a substantial number of cases. These patterns have been

observed in IA among patients with lower degree of immunosuppression (i.e. non-neutropenic and/or solid-organ transplant patients) [44-46]. Our data suggest that these non-specific signs are also frequently observed in cancer neutropenic patients. Actually, any radiological abnormality should raise the possibility of IA in these high-risk patients and trigger bronchoscopy to confirm or exclude this diagnosis among others.

Several limitations of this study should be mentioned, such as its retrospective design and the low number of cases despite 10 years of study period. Important strengths should also be outlined. First, this study was conducted in a very homogenous population for which the diagnostic approach was highly standardized. A particularity of our approach is the preemptive use of serial GM monitoring in serum and the crucial role of bronchoscopy, which was performed in 86% of pulmonary IA. Second, all CT-scan of these patients were retrospectively analyzed by a single expert in pulmonary radiology using a systematic approach (clinical report forms) allowing to obtain a rigorous description of these lesions.

In conclusion, our analysis highlights the important role of CT-scan, which was the first indicator of IA (preceding GM positivity or other positive results) in 67% cases of this study. Moreover, we showed that multiple radiological patterns, in addition to the classical nodular presentation, may be observed in pulmonary IA in this population. A low threshold should be maintained to perform chest CT-scan during the neutropenic phase (e.g. in case of persistent or relapsing fever).
We showed that the yield of galactomannan monitoring in serum was relatively modest. This strategy was useful for the early detection of 33% of cases. Because mortality rate remained high among these patients, we did not observe a significant benefit in terms of improved outcome to support this approach. Instead of monitoring GM twice per week, we may propose an alternative approach, which consists in performing punctual serum GM testing at crucial points during the neutropenic phase (e.g. persistent or relapsing fever).

Finally, our strategy supports the use of bronchoscopy in case of any abnormal lung lesion on chest CT among these high-risk neutropenic patients, as this invasive procedure was actually required to document half of the IA cases in this series.

Output

These results were presented as a poster (P224) at the international meeting *Trends in Medical Mycology* (6-9 October 2017, Belgrad, Serbia). The abstract has been published with all abstracts of this meeting in a supplement issue of the Journal *Mycoses* (2017, 60, suppl 2; 53-238)

An original research article has been written and will be submitted soon for publication in an international peer-reviewed journal.

Further analysis focusing on the radiological patterns of IA is still ongoing and will make the object of another original research manuscript.

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Tables

Table 1. Characteristics of invasive aspergillosis (IA) cases (N=30)

Hematologic malignancy	
Acute myeloid leukemia	21 (70%)
Acute lymphoid leukemia	4 (13%)
Myelodysplastic syndrome	4 (13%)
Aplastic Anemia	1 (3%)
EORTC-MSG classification	
Proven / probable	6 (20%) / 24 (80%)
Primary site of infection	
Lung	28 (93%)
Sinus	1 (3%)
Brain	1 (3%)
Secondary sites of infection[1]	2 (7%)
Pathogen	
Aspergillus fumigatus	14 (47%)
Other *Aspergillus* spp.[2]	3 (10%)
Not documented[3]	13 (43%)
Non-fungal infections[4]	10 (33%)

[1] Sinus, skin

[2] *A. flavus*, *A. terreus*, *A. glaucus* / *A. oryzae* (mixed)

[3] Diagnosis based on positive galactomannan only

[4] concomitant microbiologically documented bacterial or viral infections

Table 2: Respective contribution of each diagnostic marker of invasive aspergillosis (N=30)

Diagnostic marker	N (%)
GM only	**13 (43%)**
GM serum only	6 (20%)
GM BAL only	3 (10%)
GM serum and BAL	4 (13%)
GM and other[1]	**13 (43%)**
GM and PCR	11 (37%)
GM and culture	2 (6%)
Other than GM only	**4 (13%)**
PCR only	1 (3%)
Culture only	2 (6%)
PCR and culture	1 (3%)

GM = galactomannan, BAL = bronchoalveolar lavage fluid, PCR = *Aspergillus*-specific and/or panfungal polymerase chain reaction

Table 3: Stratification of IA cases (N=30) according to first diagnostic marker

Groups	First IA marker	Confirmation	N (%)	Response to therapy (w4)	Survival (w12)
GM	Serum GM	CT	10 (33%)	5/9 (56%)[2]	6/10 (60%)
CT	CT	Serum GM	5 (17%)	2/4 (50%)[2]	2/4 (50%)[2]
IP	CT	Bronchoscopy or other IP [1]	15 (50%)	15/15 (100%)	15/15 (100%)

GM = galactomannan, CT = computed tomography, IP = invasive procedure (e.g. tissue biopsy), w4 and w12: week 4 and 12 from start of antifungal therapy, respectively.

[1] Bronchoscopy only in 10 cases, both bronchoscopy and tissue biopsy (lung, sinus) in 4 cases, tissue biopsy only (brain) in one case.

[2] One patient was lost for follow-up and one patient could not be assessed for response to therapy at week 4.

Table 4. Radiological signs of pulmonary invasive aspergillosis (N=28)

Number of lesions	
Single	4 (14%)
2 – 5	12 (43%)
5 – 10	5 (18%)
> 10	7 (25%)
Nodules	**24 (86%)**
< 3mm	1 (4%)
3 – 10 mm	8 (33%)
> 10 mm	14 (58%)
> 30 mm	1 (4%)
Halo sign	21 (88%)
Air-crescent sign	0 (0%)
Cavity	2 (8%)
Other CT lesions	
Tree-in-bud	5 (18%)
Alveolar condensation	21 (75%)
Ground-glass opacity	11 (39%)
Non-specific density	19 (68%)
Pleural effusion	10 (36%)